

B1
cont'd

sperm DNA, and 600 μ M biotinylated DNA, 26-mer. The probe sequence was 5'CGCTAATATCAGAGAGATAACCCAC-3' (SEQ. ID NO.1). Wafers were incubated in this solution overnight at 4°C. Wafers were removed from the solution and washed with 1x phosphate buffered saline containing 0.2% Tween 20™ detergent (PBS/Tween). The wafers were coated in a BSA (bovine serum albumin) solution for 3 hours at 65°C. There wafers were then rinsed with PBS/Tween detergent.

Please replace the paragraph starting on Line 19 on Page 40 with the following amended paragraph. The changes to this paragraph are shown explicitly in the attached "Version with Markings to Show Changes Made."

B2

Wafers coated with T-polymer (see Example 1) were coated for 56 hours at 4°C in a solution containing 50 mM sodium citrate buffer, pH 6.0, 5X SSC, and 20 μ g/ml of the ssDNA capture probe complimentary to M13mp18. The probe sequence was CGCTAATATCAGAGAGATAACCCAC (SEQ. ID NO. 1). Probe coated wafers were removed from coating solution and placed into a blocking solution containing 5X Denhart's solution, 0.5% SDS, 1mg/ml carrier DNA, and 25mM buffer at pH 6.5. They were incubated 16-18 hours at 4°C and then rinsed with phosphate buffered saline containing 0.0005% TWEEN20 detergent at pH 7.4. Capture probe coated wafers were hybridized with M13mp18 plasmid overnight at 60°C in a solution containing 1X Denhardt's solution, 0.5% SDS, 25 mM MES, pH 6.5, 0.2 mg/ml carrier DNA, 5X SSC, a final concentration of M13mp18 was 500ng/ml, 1 ng/ml or 100pg/ml. The final hybridization step occurred under the same solution and incubation conditions as the previous step with a final biotinylated amplifying probe concentration of 92 μ M. The amplifying probe contains strand sequence from 6249 to 6273 and was biotinylated at residue 6261. The sequence is GCAGGTCGACTGTAGCAGGATGCCGG (SEQ. ID NO. 2). All appropriate controls were performed. Wafers were incubated with a streptavidin alkaline phosphatase conjugate. Precipitating substrate, BCIP/nitroblue tetrazolium, was used to generate an increase in thickness at the surface of the wafer. Thickness increases were measured using an absolute ellipsometer (Gaertner). Results for the experiment are shown in Figure 2. From this experiment, it was concluded that

B2
cont'd

a sensitivity of 1 ng/ml and potentially as low as 100 pg/ml was achieved. This translates to a copy number of roughly 10 10 for a very un-optimized assay.

Please replace the paragraph starting on Line 29 on Page 43 with the following amended paragraph. The changes to this paragraph are shown explicitly in the attached "Version with Markings to Show Changes Made."

B3

An 18 mer DNA/RNA chimera was utilized as the capture probe. The probe was biotinylated at the 5' end and has a ribonucleotide cytosine on the 3' end. The DNA sequence was 5'-CGAAGCTTGGATCCGCC-3' (ribose) (SEQ. ID No. 3). The covalently attached capture probe was treated with S1 nuclease to degrade the entire probe from the surface. The S1 nuclease was mixed in a solution of 0.2 mM NaCl, 0.05 M sodium acetate pH 5.4, 1 mM ZnSO₄, and 0.5% glycerol to a final concentration of 2 units/ml. A section of the wafer was submerged into 7 ml of the enzyme solution and incubated for 10 minutes at 37°C. Wafers were rinsed prior to enzyme treatment in water for 2 hours at 45°C. The enzyme solution was decanted into test tubes and a small volume of water used to rinse the wafers. The combined solution was dried with a SpeedVac system. The pellet was extracted into acetone and the solution dried. These pellets were re-suspended in 70 µl of water and the A₂₆₀ measured in a microcuvette. The surface density of the probe was determined to be 50 ng/cm². Control surfaces where no NaIO₄ was used, no covalent attachment, did not generate signal. Control surfaces without DNA or without S1 nuclease gave no signal.

Please replace the paragraph starting on Line 30 on page 44 with the following amended paragraph. The changes to this paragraph are shown explicitly in the attached "Version with Markings to Show Changes Made."

B4

An 8-mer was synthesized and was 5' biotinylated and had a 3' ribose. The DNA sequence was 5'AAAGATGTA (ribose)-3' (SEQ ID NO. 4). The 8-mer was immobilized using the 15:1 periodate:probe ratio to a TC7 coated optical substrate as